Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Hematology, Serum Total Cholesterol and Thyroid Hormone Concentrations in Cyclic and Acyclic Nili-Ravi Buffaloes

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ARTICLE HISTORY (16-242)

Received: September 22, 2016
Revised: October 21, 2016
Accepted: November 04, 2016
Published online: December 21, 2016

Key words:
Acyclic
Buffalo
Cyclic
Hematology
Serum cholesterol
T3 and T4

A B S T R A C T

In this study, hematological variables and serum levels of total cholesterol and thyroid hormones (T_3 and T_4) in cyclic and acyclic Nili-Ravi buffaloes were investigated. Sixty buffaloes with clinically normal reproductive tract were divided into two equal groups i.e. cyclic and acyclic, depending upon the presence or absence of active corpus luteum on the ovaries. Blood samples with and without anticoagulant were collected aseptically from each animal. Serum was harvested from blood samples without anticoagulant and utilized for estimation of total cholesterol, T3 and T4 concentrations, using kit method. Blood samples containing anticoagulant were used for determination of hematological variables viz. Hb, PCV, ESR, MCV, MCH, MCHC, TEC, TLC, DLC and platelets count. Mean values of total cholesterol (142.85±7.43 vs 88.84±5.33 mg/dl), Hb (11.54±1.61 vs 9.87±1.14 g/dl), PCV (40.28±6.06 vs 36.80±4.30%), MCV (64.21±3.55 vs 56.81±5.35fl), MCH (21.58±5.47 vs 15.99±1.84 pg), MCHC (29.32±2.52 vs 26.95±2.0 g/dl) and TEC (6.29±0.97 vs 4.87±1.62x10^6/µl) were higher in cyclic than acyclic buffaloes ($P\leq0.05$), while reverse was true for TLC and platelet counts. However, ESR, lymphocyte, monocyte, eosinophil and neutrophil percentages did not differ between animals of the two groups. Moreover, mean values of T3 and T4 in cyclic and acyclic buffaloes did not differ. In conclusion, low level of serum cholesterol might have been among causes of anestrous in these buffaloes, as cholesterol is the precursor of sex hormones like progesterone and estradiol, and its level was lower in acyclic than cyclic buffaloes. However, thyroid hormones do not seem to play any significant role in the occurrence of this problem.

To Cite This Article: Ghani MU, Ahmad I, Ahmad N, Ijaz N and Mehfooz A, 2017. Hematology, serum total cholesterol and thyroid hormone concentrations in cyclic and acyclic Nili-Ravi buffaloes. Pak Vet J, 37(1): 31-34.

INTRODUCTION

Regular reproductive cyclicity is of prime importance for profitable dairy farming. Endocrine system plays an important role in controlling the reproductive and other body functions (Pineda and Michael, 2003). Thyroid hormones, triiodothyronine (T3), thyroxin (T4) and calcitonin (CT) are related with maintenance of basal metabolic rate and thus indirectly influence reproductive function both in the male and the female.

Thyroid disorders are more common in female than in male animals. Hypothyroidism results in irregular or cessation of estrous cycle with reduced conception rate. Low levels of T3 and T4 are also associated with delayed puberty (Pamela *et al*., 2009). Similarly, cholesterol performs multiple functions in the body and is structural component of the cell membrane. It is also precursor of all classes of steroid hormones including glucocorticoids (cortisol), mineralocorticoids (aldosterone), sex hormones (androgens, estrogens, progestins) and Vitamin-D. Utilization of cholesterol is related to the ovarian functional activity. The serum cholesterol levels have been reported to be low in acyclic compared to cyclic postpartum Surti buffaloes (Parmar *et al*., 2015). A significant negative relationship has also been reported between concentrations of thyroid hormones, total lipids (TL) and total cholesterol in cattle (Saleh *et al.*, 2011).

Similarly, the knowledge of hematological variables is useful in diagnosing various pathological and metabolic disorders, which can adversely affect the productive and reproductive performance of dairy animals, resulting in great economic losses to dairy farmers (Dutta *et al.*, 1991). Changes in hematological constituents are important indicators of the physiological or pathological state of the animal.

Pakistan is blessed with some of the fine dairy breeds of buffaloes namely, Nili-Ravi and Kundi, but their potential is not fully exploited. Buffalo is a polycyclic animal which breeds throughout the year except during hot summer months (Qureshi *et al.*, 1999), when sexual activity of this species is suppressed. During this period, most of the buffaloes remain sexually dormant without any sign of estrus. This condition is generally known as summer anestrous and is characterized by inactive ovaries with disturbed hormonal profiles. Besides summer, anestrous also occurs during other seasons, with its incidence varying from 25.84 to 40.14% (Kumar *et al.*, 2013), and this condition may be associated with thyroid activity and serum total cholesterol. However, there is relatively little information regarding possible association of this problem with serum cholesterol and thyroid activity in Nili-Ravi buffaloes.

Therefore, the present study was planned to investigate serum total cholesterol and thyroid hormones profile in cyclic and acyclic Nili-Ravi buffaloes. Attempts were also made to monitor changes in hematological variables among animals of the two groups.

MATERIALS AND METHODS

Selection of animals: A total of 60 adult Nili-Ravi buffaloes with clinically normal reproductive tract, maintained at the Livestock Farm, University of Agriculture, Faisalabad (UAF) and those visiting the outdoor clinic of the Department of Theriogenology, UAF for treatment of reproductive problems during the period from March, 2013 to June 2013 were included in this study. In order to ascertain reproductive status, these buffaloes were examined through rectal palpation. Depending upon the ovarian status, these animals were divided into two groups i.e. cyclic and acyclic, with 30 animals in each group. Buffaloes having an active corpus luteum (CL) on any ovary were categorized as cyclic, while those having inactive ovaries (no active CL) were taken as acyclic (Kausar *et al.*, 2013).

Blood collection: About 15 ml blood was collected from each animal through jugular venepuncture. Immediately after collection, the blood was divided into two portions: one was allowed to clot, serum was separated and stored at -20℃ for further analysis, while the other part was used for hematological study after addition of an anticoagulant (EDTA at the rate of 1mg/ml).

Serum analysis: Serum concentrations of tri-iodothyronine (T3) and thyroxin (T4) were determined by using enzyme immunoassay kit (BioCheck, Inc. USA), following the protocols given by the manufacturer of the kit. After processing samples and standards, their absorbance was recorded. The concentrations of T3 or T4 in samples were taken from the standard curve plotted for absorbance of standards against their concentrations. Standards for T3 had concentrations of 0.0, 0.5, 1.0, 2.5, 5.0 and 10.0 ng/ml, while T4 standards had concentrations of 0, 2, 5, 10, 15 and 25 µg/dl.

Serum total cholesterol contents were measured through enzymatic end point method, using cholesterol kit (Catalogue No. CH-200, Randox). After processing samples and standard as per guidelines of manufacturer of kit, absorbance of samples and standard was recorded against reagent blank at 546 nm wavelength. The concentration of total cholesterol in samples was calculated through dividing absorbance of the sample by absorbance of the standard and multiplying by concentration of the standard (Ahmad *et al*., 2012). The concentration of total cholesterol in the standard was 200 mg/dl.

Hematological analysis: Blood samples with anticoagulant were used for determination of various hematological variables including hemoglobin concentration (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC) and platelets count, using standard procedures. An automatic Hematology Analyzer (Mindray-300) was used for this purpose.

Statistical analysis: Mean±SD for various quantitative variables for cyclic and acyclic buffaloes were calculated. In order to ascertain the magnitude of variation in these variables between buffaloes of the two groups, the data were subjected to t-Test through applying computer software SPSS for Windows (SPSS Inc., Version14.1).

RESULTS

Mean±SD for various serum biochemical constituents, hematological variables, total and differential leukocytic counts are shown in Table 1. Moreover, ranges for normal reference values for most of the parameters reported in the literature are also given in Table 1.

Serum biochemical constituents: Differences in serum thyroid hormone (T3 and T4) concentrations between cyclic and acyclic buffaloes were statistically non-significant. However, serum total cholesterol level was significantly higher ($P<0.05$) in cyclic than in acyclic Nili-Ravi buffaloes.

Hematological variables: Among hematological variables, mean values for blood Hb concentration, PCV, MCV, MCH, MCHC and TEC were significantly ($P\leq0.05$) higher in cyclic buffaloes compared to acyclic ones. However, values for ESR differed non-significantly between animals of the two groups. Among leukocytes, acyclic buffaloes showed significant increase in TLC and platelets counts compared to cyclic animals ($P<0.05$). However, there was no difference in differential leukocytic counts (neutrophil, lymphocyte, monocyte and eosinophil) between animals of the two experimental groups. Furthermore, the mean values recorded for most of the variables of cyclic and acyclic buffaloes (except total cholesterol and MCHC) were within the normal reference ranges reported in the literature for lactating buffaloes (Table 1).

Table 1: Hemato-biochemical variables in cyclic and acyclic Nili-Ravi buffaloes

Parameters	Variables	Cyclic buffaloes	Acyclic buffaloes	Reference ranges*
Serum constituents	Total cholesterol (mg/dl)	142.85±7.43[b]	88.84±5.33[a]	27.9-101.9
	Triiodothyronine (ng/dl)	1.81±1.24[a]	1.60 ±1.26[a]	-
	Thyroxin (µg/dl)	23.77±7.84[a]	22.81±7.27[a]	-
Hematology	Hemoglobin concentration (g/dl)	11.54±1.61[a]	9.87±1.14[b]	9.5-14.5
	Packed cell volume (%)	40.28±6.06[a]	36.80±4.30[b]	27.0-44.0
	Erythrocyte sedimentation rate (mm/hr)	77.6±4.4[a]	85.8±4.4[a]	-
	Mean corpuscular volume (fl)	64.21±3.55[a]	56.81±5.35[b]	47.0-62.0
	Mean corpuscular hemoglobin (pg)	21.58±5.47[a]	15.99±1.84[b]	15.0-21.0
	Mean corpuscular hemoglobin concentration (g/dl)	29.32±2.52[a]	26.95± 2.0[b]	31.0-35.8
	Total erythrocytic count (10^6/µl)	6.29±0.97[a]	4.87±1.62[b]	5.2-8.4
Total and differential leukocytic count	Total leukocytic count (10^3/µl)	8.6±2.0[b]	10.05±3.1[a]	6.0-12.8
	Neutrophil (%)	43.60±7.64[a]	46.57±9.52[a]	24.0-61.4
	Lymphocyte (%)	48.10±9.14[a]	50.17±8.55[a]	31.7-70.0
	Monocyte (%)	4.13±1.53[a]	4.10±1.35[a]	Up to 8.0
	Eosinophil (%)	2.1±0.92[a]	1.9±0.71[a]	Up to 10.0
	Platelet count (10^5/mm^3	365±173[b]	473±218[a]	-

Values (mean±SD) with different superscripts within a row differ significantly from each other (P<0.05); *Source: Abd-Ellah *et al.* (2014).

DISCUSSION

Blood biochemical metabolite profiles have direct influence on the reproductive performance of animals, including buffaloes, either through stimulation of hormone synthesis and release, modulating hormone action, or modifying response of target tissues through serving as precursor for synthesis of specific hormones (Parmar *et al*., 2015). In the present study, serum total cholesterol was significantly (P≤0.05) higher in cyclic compared to acyclic buffaloes. Ali and Shukla (2012) also recorded higher serum total cholesterol in cyclic than in acyclic buffaloes (132.49±3.39 mg/dl vs 75.32±1.92 mg/dl). Similar trend was reported by Amanullah *et al.* (1997) and Kabir *et al.* (2001). According to Parmar *et al.* (2015), serum total cholesterol, which was low in acyclic postpartum buffaloes, increased when estrus was induced by equine chorionic gonadotropin (eCG) and norgestomet treatment.

Estrogens have been shown to influence pituitary-thyroid-adrenal axis (Parmar *et al*., 2015). Kumar *et al.* (2015) recorded higher serum estrogens levels in estrous buffaloes compared to anestrous animals (105.84±34.12 vs 45.70±5.45 pg/ml), which stimulate carbohydrate metabolism, increasing cholesterol synthesis from acetate. Moreover, estrogens also stimulate lipid metabolism through lipogenesis, which in turn causes increased production of cholesterol. So, high cholesterol in cyclic buffaloes compared to acyclic animals might be due to higher estrogen levels in cyclic animals compared to acyclic ones. Conversely, since cholesterol is the precursor of all steroid hormones, low serum cholesterol would result in anestrous through decline in synthesis of sex steroids including estrogens. Moreover, anestrus in postpartum Murrah buffaloes has also been associated with low antioxidant status of these animals (Ghosh *et al*., 2015). Unfortunately, antioxidant status of buffaloes included in this study could not be monitored.

Thyroid gland plays an important role in regulating general metabolism, growth and development of the body. Thyroid hormones, T3 and T4, regulate energy balance in the body and thus help in maintaining physiological reproductive activity in mammals (Mayahi *et al*., 2014). Kumar *et al.* (2010) recorded significantly higher T3 and T4 levels in cyclic buffaloes, compared to acyclic ones. However, in the present study, serum T3 and T4 did not differ between cyclic and acyclic buffaloes, and its explanation remains unclear. It is possible that thyroid hormones do not play any significant role in causing the problem of acyclicity in buffaloes, although these hormones regulate body metabolism and can influence onset of puberty. However, further studies involving antioxidant status are suggested to see whether thyroid activity has any association with acyclicity in Nili-Ravi buffaloes.

The pattern of blood Hb concentrations of cyclic and acyclic buffaloes recorded in this study corroborates with the findings of Ali and Shukla (2012), who recorded higher Hb values (12.63±0.49 vs 9.81±0.21 g/dl) in cyclic than in acyclic buffaloes. Similarly, Kumar *et al.* (2015) recorded blood hemoglobin level of 13.34±0.43g% in estrous buffaloes compared to 11.38±0.56g% in anestrous buffaloes (P<0.05). These workers also observed higher levels of total proteins in estrous buffaloes compared to anestrous ones. Hemoglobin is a conjugated transport protein and its higher levels in estrus animals may be due to increased synthesis of proteins in these buffaloes. Moreover, the present study was conducted during March to June, when the ambient temperature is high and blood Hb concentration and PCV have been shown to increase in young and adult buffaloes with increase in temperature (Haque *et al*., 2013). A low level of blood hemoglobin in acyclic buffaloes can suppress tissue oxygenation of the reproductive tract, which in turn would affect the ovarian activity (Ramkrishna, 1997).

Higher PCV recorded in cyclic buffaloes can be attributed to good nutritional status and higher concentrations of estrogens, which are known to have anabolic activity. The low PCV in acyclic buffaloes is suggestive of malnutrition in these animals. Earlier, Islam *et al.* (1999) made similar observations in cross-bred cows from Bangladesh. In the present study, MCV was low in acyclic animals, which is suggestive of iron deficiency that might have resulted from some sub-clinical chronic disease, which escaped detection during selection of the animals. The MCH and MCHC have been shown to vary with Hb synthesis. Blood hemoglobin concentration in this study was higher in cyclic group, which could have resulted in higher MCH and MCHC in cyclic than acyclic buffaloes.

The RBC count is an index of health status of an individual. High RBC count in cyclic animals may be due to excitement and hyperactivity under the influence of estrogens. Total leukocyte count, as well as platelets count, was significantly ($P\leq0.05$) higher in acyclic as compare to cyclic group. The mean values of total leukocyte count in this study are in line to those reported by Patil *et al.* (1992) and Horadagoda *et al.* (2002), and are also within the reference range of 6.0-12.8 x 10^3/µl (Table 1) reported by Abd-Ellah *et al.* (2014). The significantly higher values of total leukocyte count and platelets count in acyclic group are suggestive of some subclinical problem (Connell *et al.*, 2008). Animals included in the study did not show any clinical abnormality; however, the presence of some sub-clinical infection in these buffaloes cannot be ruled out. This speculation is supported by the fact that buffaloes of both experimental groups showed lower MCHC than reference range and apparently high ESR. Moreover, mean values of TEC in acyclic buffaloes were slightly lower than their reference range (Table 1). Animals included in this study belonged to private livestock owners and were reared under divergent nutrition and management which could have influenced their blood picture. The exact reason for increased TLC and platelets count in acyclic buffaloes and its physiological significance remains to be further explored.

The neutrophil, eosinophil, monocyte and lymphocyte percentages did not differ between cyclic and acyclic buffaloes. These findings are in agreement with those of Ali and Shukla (2012).

Conclusions: Based on the results of this study it can be concluded that low level of serum cholesterol might have been among causes of anestrous in these buffaloes, as cholesterol is the precursor of sex hormones like progesterone and estradiol, and its level was lower in acyclic than cyclic buffaloes. However, thyroid hormones do not seem to play any significant role in the occurrence of this problem.

Authors contribution: This manuscript is based on MPhil thesis of the 1st author. IA and NA conceived the idea and prepared the manuscript. MUG, conducted the experimental work, while NI and AM helped in sample collection and statistical analysis of the data. All authors approved the contents of manuscript.

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